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THE DIAGNOSIS OF HUMAN PULMONARY HYDATIDOSIS BY THE IMMUNOELECTROPHORESIS TEST

LUIS A. YARZÁBAL,* JUDITH LEITON, AND MARÍA H. LÓPEZ-LEMES
Laboratorio de Inmunología Parasitaria, Hospital de Clinicas, Facultad de Medicina, Casilla de Correo 1737, Montevideo, Uruguay

Abstract. A specific and characteristic precipitation band appeared in immunoelectrophoresis tests with sera from hydatidosis patients, when fluid from either fertile or sterile bovine liver cysts of *Echinococcus granulosus* was used as antigen. No "false positives" were found using this test on sera from normal subjects and from patients with tuberculosis, aspergillosis, acute pneumonia, hepatic cirrhosis or pulmonary carcinoma. The immunoelectrophoresis test showed a higher sensitivity than the indirect hemagglutination test in the preoperative diagnosis of pulmonary hydatidosis in 54 patients whose infections were subsequently confirmed at surgery. It was possible to classify hydatid cysts into four categories based upon the physical integrity of cyst membranes observed at surgery and the histopathological response of the host. In individuals having recently broken cysts the immunoelectrophoresis test was uniformly positive whereas in those with only remnants or unbroken cysts, the proportion of positive reactors was markedly less. It is postulated that the physical status of the hydatid cyst membranes influences the degree of antigen stimulation of the immune system of the host and therefore affects the success or otherwise of immunodiagnostic tests.

Human infection with *Echinococcus granulosus* reaches possibly its greatest prevalence in the countries of southern South America.[1,2] The clinical incidence in Uruguay, a country with an estimated population of 2,851,600 inhabitants,[3] is of the magnitude of more than 500 cases per year,[4] and serological tests are fundamentally important in the successful diagnosis of these infections. However, variations in the sensitivity and specificity of serological tests necessitate the use of two or more such methods in the diagnosis of hydatid disease.

In 1967, Capron et al.[5] reported the presence of an antigen specific for *E. granulosus* in hydatid cyst fluid from horses which appeared in immunoelectrophoresis (IEP) as a band of characteristic morphology and localization when tested against sera from human patients. They termed this band "arc 5" because of its relative position in the immunoelectrophoretic pattern.

In the present study the immunoelectrophoretic test was used in the diagnosis of 111 cases of pulmonary hydatidosis. Both fertile and infertile bovine cyst fluids were used successfully as sources of specific antigen. In sera from 54 of these patients studied simultaneously by the IEP and indirect hemagglutination (IHA) tests, the IEP test showed a greater overall specificity and sensitivity. Complete clinical, surgical and pathological records were available for each patient and the cysts were classified according to the physical integrity of the parasite membranes and the histopathological reaction in the surrounding tissues. Observations are presented on the association between serological reactivity and cyst classification according to these criteria.

MATERIALS AND METHODS

The 111 individuals selected for this study were patients at the Instituto del Tórax, Colonia Saint-Bois Hospital, Montevideo, Uruguay between January 1969 and January 1971. They had been referred to this hospital by physicians throughout the country and in each case the clinical and radiological diagnoses were compatible with pulmonary hydatidosis. Recent clinical histories were taken and sera were collected during the preoperative period and stored at −20° C. The diagnosis was confirmed in all cases by surgery performed at the same hospital. None of the hydatid cysts were fertile and no brood capsules were observed in any of these cases. The possibility of simultaneous hepatic infection was explored by

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* Present address: Instituto Bacteriologico de Chile, Marathon 1000—Casilla 48, Santiago, Chile.

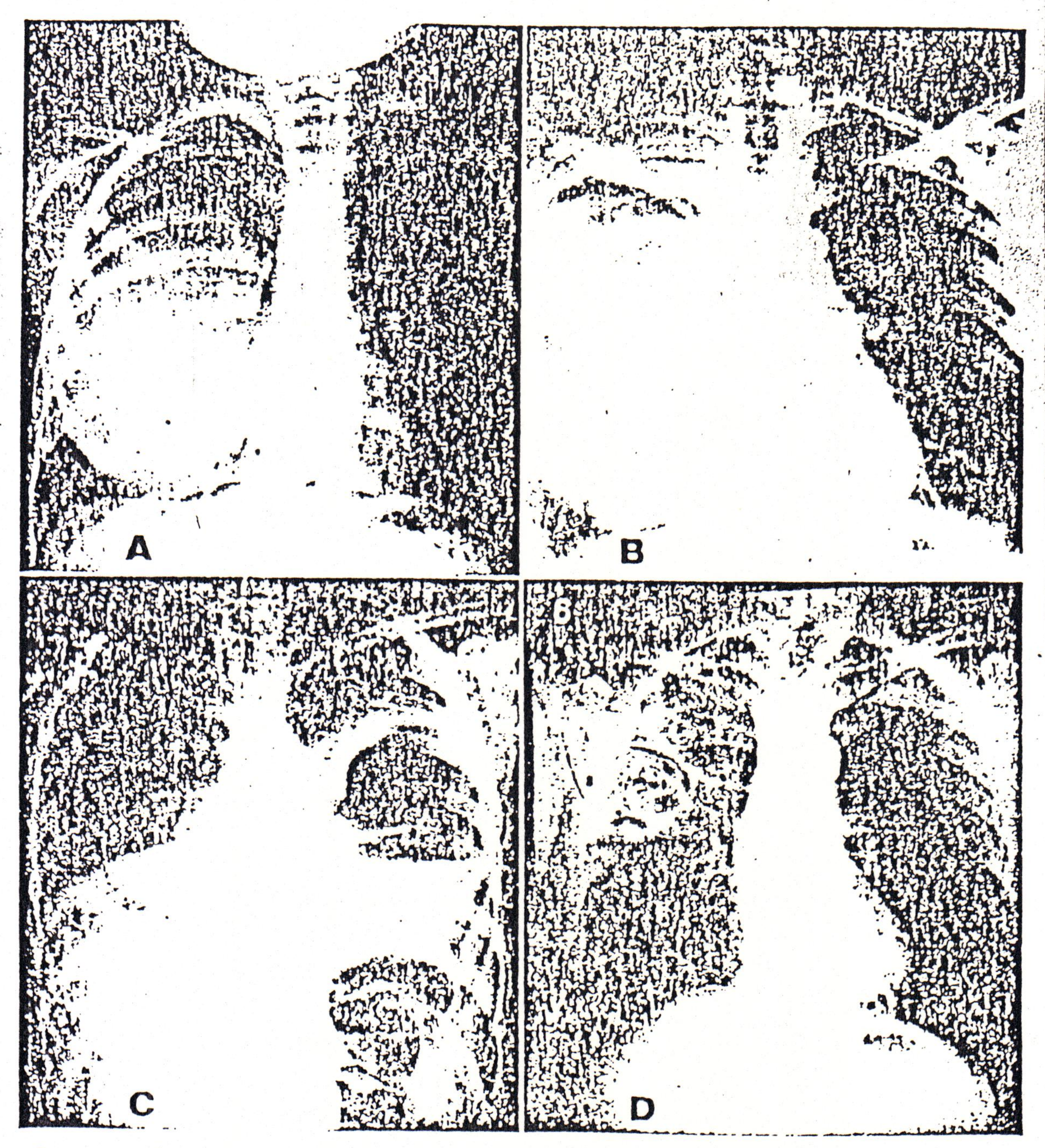


FIGURE 1. Chest radiographs. A, shows hyaline hydatid cyst in right lung; B, shows infected hydatid cyst in right lung. The crescent-shaped image results from the infiltration of air between the host capsule and the parasite-membranes. At surgery this space was found to contain a small amount of purulent fluid; C, shows recently broken hydatid cyst in left lung. An area of pneumonitis is observed above the cavity and the parasite membranes are suspended in the fluid within; D, shows a broken hydatid cyst containing the remnants of the parasite. No hydatid fluid was present.

means of complete clinical examination in all 111 patients and by hepatic gammagram* tests in 24 cases. No hepatic infection was revealed by these procedures.

Patients were divided into groups according to the characteristics of their hydatid cysts at surgery. The cysts were classified by means of

* Performed at the "Centro de Medicina Nuclear. Clinica Medica A. Facultad de Medicina, Montevideo, Uruguay."

macroscopic and histological examination as follows: 1) hyaline—when the parasite membranes were found to be macroscopically unaltered and the hydatid fluid within was clear and transparent (Fig. 1A); 2) infected—when there was evidence of suppurative infiltration between the parasite bladder and the host capsule, and or turbidity of hydatid fluid, without an apparent rupture of the cyst membranes (Fig. 1B); 3) recently broken—when the membranes were recently torn (at least 6 months before blood collection) and were found floating or immersed in the fluid which partially occupied the cavity limited by the host capsule (Fig. 1C). The cyst fluid in these cases was often purulent, opaque, and hemorrhagic; 4) remnants—when the remains of the parasite membrane were retained within the host capsule in the absence of hydatid fluid which was either reabsorbed or expelled through the respiratory tract (Fig. 1D). The clinical history of these patients indicated that symptoms related to cyst rupture had occurred at some time before the 6 months preceding blood collection.

In some individual patients hyaline cysts were found in juxtaposition to cysts fitting the description of the three other categories. When the final results were tabulated these patients were classified as belonging to the pertinent non-hyaline group. When one cyst belonged to group 4 (remnants) and the other(s) to categories 2 and or 3, they were included with the latter. The "recently-broken" criterion was considered crucial and prevailed over both "remnants" and infected categories. The basis for this classification was that antigenic stimulation (reflected by positive serology or single-cyst infections) was observed to decrease in the following order: group 3, 2, 4, and 1. In no case were infected cysts found in the presence of another fitting the "remnants" category.

The hydatid fluid used as antigen was collected from bovine liver cysts and several batches were prepared by pooling the fluid obtained from all the cysts in each liver. These pools were dialyzed, lyophilized and standardized as described previously.[5] Only those batches which contained at least 6 parasite antigens, including the characteristic "arc 5," and 4 or less host antigens, as revealed by immunoelectrophoretic analysis against a rabbit antibovine liver serum, were selected for use in the diagnostic tests. Since preliminary

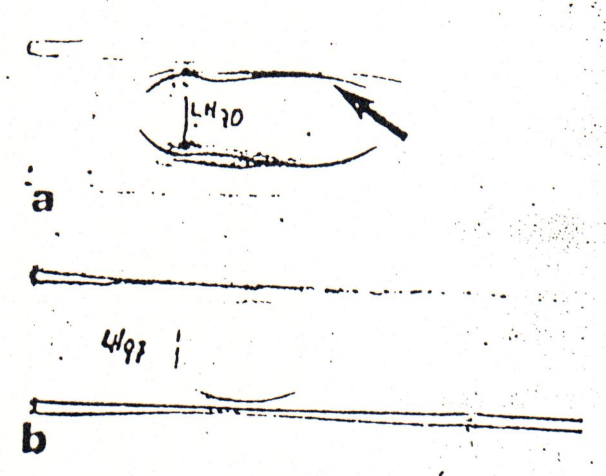


FIGURE 2. A, presence of the diagnostic arc 5 in the sera of two hydatidosis patients by immunoelectrophoresis (IEP). Center well contains whole bovine hydatid fluid. Patient's sera is in the lateral troughs. Anode to right of picture; B, unidentified arcs revealed by IEP in the sera of two pulmonary hydatidosis patients. Upper trough contains serum from a patient with parasite remnants; lower trough contains serum from a patient with a hyaline cyst; center well contains bovine hydatid fluid. Anode to right of picture.

studies demonstrated that the *E. granulosus* specific antigen was found in fertile and sterile bovine liver cysts no effort was made to separate the two.

Immunoelectrophoretic analysis was performed on 7.5 cm × 2.5 cm slides according to the method of Capron et al.[5] Agarose was used at 0.9% and the pH of the buffer system was 8.2.

The patient's serum was lyophilized, reconstituted to ⅓ of the original volume and placed in the troughs after the antigen (at a concentration of 200 mg dry wt ml) was subjected to electrophoresis for 1.5 hours. Precipitation bands were allowed to develop over a period of 48 hours. Patients whose serum reacted with the production of the specific "arc 5" were diagnosed as having hydatid disease and the results were subsequently compared with the surgical findings.

The diagnostic specificity of these hydatid antigens was evaluated by using sera from 100 normal subjects and from 100 patients with tuberculosis, 40 with broncho-pulmonary cancer, 30 with hepatic cirrhosis, 20 with aspergillosis, and 10 with acute pneumonias of diverse etiology.

The sera from 54 patients with pulmonary hydatidosis were also examined by the hemag-

TABLE 1

Results of immunoelectrophoretic analysis of sera from 111 cases of pulmonary hydatidosis

Classification of the cysts	No. cases	Positive (%)
Hyaline	42	17 (40.4)
Infected	3	2 (66.6)
Recently broken	55	51 (92.7)
Remnants	11	5 (45.4)
Total	111	75 (67.5)

TABLE 2

Results of immunoelectrophoresis (IEP) and Indirect hemagglutination (IHA) tests in 54 cases of pulmonary hydatidosis

Classification of the cysts	No. cases	IEP positive	IHA positive
Hyaline	28	10	8
Infected	2	2	2
Recently broken	14	14	14
Remnants	10	8	6
Total	54	34 (62.9%)	30 (55.5%)

glutination test of Garabedian et al.,[7] using sheep erythrocytes sensitized with the same antigen used for the immunoelectrophoresis test.

RESULTS

The antigen(s) responsible for the formation of the *E. granulosus* specific "arc 5" has been found in hydatid fluid from both fertile and sterile bovine liver cysts. Using the development of arc 5 as the diagnostic criterion no "false positive" reaction was obtained with sera from patients suffering from tuberculosis, aspergillosis acute pneumonia, hepatic cirrhosis or pulmonary carcinoma, or from normal persons.

The characteristic "arc 5" appeared with sera from 75 patients with clinical and radiologic findings suggestive of pulmonary hydatidosis subsequently confirmed at surgery. A slide representative of a positive result is shown in Figure 2A. Sera from the remaining 36 patients yielded negative results, although uncharacterized bands (Fig. 2B) were observed in 10 cases.

The relationship between the characteristics of the parasite cysts at surgery and the results of the immunoelectrophoretic tests performed on the sera of the patients is shown in Table 1.

When the sera from 54 of the hydatid patients were examined by the hemagglutination test, 30 had titers above the 1:64 diagnostic level (determined in our laboratory) while 24 were negative (Table 2). Thirty-four of these 54 sera were positive in the IEP test.

DISCUSSION

The serologic diagnosis of hydatid disease by immunoelectrophoresis (IEP) was first described by Capron et al.[?] using hydatid fluid from fertile horse cysts. They demonstrated the presence of a specific precipitation band, which they designated arc 5, in immunoelectrophoresis tests with homologous rabbit antisera, and showed that this arc appeared in a high proportion of sera from human patients with hydatidosis.

Capron et al.[8] later evaluated the sensitivity of this technique in the preoperative diagnosis of 188 patients suffering from hydatid disease of the liver, lungs and other organs.

They found that the sera of 84% yielded positive reactions, a higher sensitivity than was obtained with other serological techniques including indirect hemagglutination. Of the 52 cases of pulmonary hydatidosis included in this group, 69% were positive to the IEP test and 60.8% to the hemagglutination test. A lower detection rate for hydatid cysts of the lung has also been reported by other authors.[8,9]

In the present study hydatid fluid from both sterile and fertile bovine cysts was found to contain the specific antigen. Preoperative sera from 67.5% of 111 surgically proven cases of pulmonary hydatidosis reacted with specific arc 5. In the group of 54 hydatid sera tested simultaneously with both IEP and IHA tests, 34 (63.9%) were positive to the IEP and 30 (55.5) to the IHA, confirming the higher sensitivity of the IEP test.[8,10] Of the 37 cases which did not develop arc 5, 10 reacted with other hydatid fluid antigens to produce uncharacterized bands of slower anodic mobility (Fig. 2B). These reactions were detected in 8 patients with hyaline cysts (group 1) and in 2 patients with retained cyst membranes without fluid (group 4). No false positive was observed with sera from normal individuals or from patients with other infectious diseases, some of which have previously been found to cross-react in tests for hydatid disease by various serodiagnostic techniques.[10,11]

Patients in whom the cyst membrane had ruptured at least 6 months prior to the collection of serum (group 3) were positive in nearly all cases in both IEP and IHA tests, and revealed the highest number of precipitin bands (Tables 1 and 2). When the rupture had occurred before this time (group 4) more serologically negative cases were observed (54.5%). The highest percentage of patients with negative serology was found among those with hyaline cysts (59.5%), suggesting that serological positivity is a function of the pathological state of the infection and the integrity of the cyst membranes. This conclusion is supported by the observation that the sera of five patients in the present study having hyaline cysts and negative serology, were positive to the IEP test 10 days after surgical intervention at which time the cyst membranes had been ruptured. Ten other patients in the same group, from whom the cysts were removed intact still had no detectable antibodies after surgery. The rapid postsurgical appearance of antibody activity may represent a secondary response to the antigens in hydatid fluid. If the embryonic stages of development and the cyst fluid share common antigens, a state of immunological memory may have been established earlier and a secondary type of response would be expected to follow leakage of these antigens from the cyst ruptured at surgery.

In a previous study on the relationship between the integrity of cyst membranes and the IEP test response of the patients, location of the cysts was not considered and infected cysts were not differentiated from those with retained membranes.[3] In the present study pulmonary cysts were readily classifiable into the four categories described. The results indicate that in order to evaluate the sensitivity of serologic tests for hydatid disease it is imperative to take into account the physical characteristics of the cysts.

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